Chitosan Production

Field of the Invention

The invention relates to producing chitosan.

Cross Reference To Related Applications

Priority is claimed to U.S. provisional application number 60/369,594, filed April 2, 2002, which is herein incorporated by reference.

Background

Chitosan is a deacetylated form of chitin. Chitin is a polysaccharide that is found in the shells of insects, crustaceans, mollusks, and fungal biomass. Chitosan has been identified as having various uses, for example as a binder in paper making, a component in bandages, and as a wound healing compound.

The quality of chitosan varies with the degree of deacetylation of the N-acetyl groups, molecular weight, purity, manufacturing process, color, clarity, consistency, and uniformity.

Summary

The invention provides a method for producing fungal chitosan from chitin-containing material using greater than 0 PSIG (pounds per square inch gauge). This method allows for the production of chitosan with increased deacetylation levels and increased molecular weight compared to similar processes that do not use increased pressure. Similarly, the invention provides chitosan that has greater purity increased molecular weight, and increased deacetylation compared to processes that do not use

increased pressure. Because the invention provides a method for producing high purity fungal chitosan from chitin-containing material it is not necessary to take additional steps to purify the chitosan. However, if the desired product requires utilization of reaction parameters that do not yield high purity it may be desirable to separate the chitosan from the reaction. Separation can be accomplished using any method known in the art, i.e filitration, centrifugation, etc.

Another aspect of the invention provides compositions made by the method.

In yet another aspect the invention provides fungal chitosan compositions that are characterized by their combination of increased molecular weight and increased deacetylation levels, as well as compositions characterized by their combination of increased deacetylation levels, increased molecular weight and increased purity.

Detailed Description

Fungal Biomass

Chitosan described herein is prepared from chitin contained in fungal biomass. Suitable sources of fungal biomass include, for example, *Aspergillus niger, Aspergillus terreus, Aspergillus oryzae, Candida guillermondii, Mucor rouxii, Penicillium chrysogenum,* and *Penicillium notatum.*

Fungal biomass usually has between 5 and 25 percent chitin, and typically from 10 to 20 percent chitin, based upon dry weight of the biomass. Particularly useful sources of fungal biomass are commercial fermentation processes such as those used to make organic acids, such as citric acid.

Caustic

Caustic material can be used either in the reaction directly or in an aqueous solution. Examples of caustic material that can be used in the reaction include,

sodium hydroxide, potassium hydroxide, calcium hydroxide, caustic alcohol, or other alkalis. Any concentration of caustic can be used provided that the caustic reacts with the other components of the reaction to yield chitosan. Generally, caustic is used at a concentration from about 5% to about 40% by weight, and more specifically from about 15% to about 30% by weight.

Reaction Conditions

The reaction that causes the production of chitosan from fungal biomass and/or chitin from fungal biomass (hereinafter collectively referred to as chitin-containing material), involves reacting the caustic material with the chitin-containing material at a pressure greater than atmospheric pressure. The temperature, time of reaction, and pressure that are used to form chitosan will vary depending on the desired deacetylation level and the desired molecular weight of the chitosan.

Any temperature that will produce the desired chitosan product can be used. However, temperatures from about 80°C to about 150°C and more specifically, temperatures greater than 90°C, 100°C, 115°C, 125°C, 130°C, and 140°C can be used to produce the chitosan.

The reaction can be carried out for any length of time that will produce the desired chitosan product. However, typical reactions times vary from about 1 hour to about 50 hours and more specifically, reaction times greater than 4, 6, 10, 15, 20, 25, and 30 hours are preferred.

Any pressure that is greater than 0 PSIG can be used to produce the chitosan. Generally, pressures greater than 1, 2, 3, 5, 10, 15, or 20 PSIG are used. The pressure can be increased to the theoretical maximum pressure, which depends on the temperature, solubility of the caustic, and the concentration of other reactants in the solution.

Pressure can be applied by using any method known to those of ordinary skill in the art. For example, pressure in the reacting vessel can come from increased vapor pressure due to higher temperatures achieved in a closed vessel, or can come from an external force applied to the vessel contents. Increasing the temperature to 130°C, in a closed vessel containing water, will increase the pressure in that vessel to approximately 15 PSIG. Another way to increase the pressure would be to maintain temperature at a constant level, and apply an outside source of pressure, by reducing the volume of the container, or attaching an outside gas source to raise the pressure to the desired level. This outside source could be an inert gas such as nitrogen, helium or ammonium from a pressurized tank.

Fungal Chitosan

The compositions of the invention are characterized by their combination of high deacetylation levels and high molecular weights. Compositions of the invention can have deacetylation levels greater than 85%, 90%, and 95%. Similarly, compositions of the invention can have molecular weights greater than 80,000, 90,000, 100,000, 150,000, and 175,000.

In other embodiments compositions of the invention can be characterized by their purity level. For example fungal chitosan composition having purity levels of greater than 85%, 90%, and 95% can be obtained.

Examples

The following examples are provided to demonstrate production of fungal chitosan from a chitin containing material. In the examples depicted, the chitosan was produced under pilot laboratory conditions. However, the invention is also applicable to production of chitosan in large-scale manufacturing operations, particularly where uniform sources of fungal biomass are available.

Example 1. Method of obtaining and purifying chitosan from fungal biomass using 20.1% NaOH at greater than 0 PSIG.

29.9 kg of fungal biomass (*Aspergillus niger)* at 17.14% dry solids, 21 liters of 50% NaOH, and 18 liters of water were added to a pressure reactor which was made using materials available on site. However, commercial models such as, for example, the Miniclave Pressure Reactor from CTP Corporation, Northport, NY, can also be used to give the results provided herein. This resulted in a final ratio in the mixture of 6.0% dry biomass, 20.1 % NaOH, and 73.9% water. This alkali biomass solution was heated using a steam coil to approximately 130°C and held in the sealed reactor for 28 hours. Since this was above the boiling point of 20% caustic (109°C), 14-16 PSIG pressure was contained in the reactor, as well as the ammonia and other gases released in the associated reactions.

Samples were taken periodically. The samples were filtered and washed with water to remove the NaOH, salts and other soluble by-products. The filtered solids contained the chitosan-containing material, made up primarily of chitosan and glucans. The chitosan was then separated from the glucans by dissolving the filtered solids in acetic acid (pH 4.0), and centrifuging to separate the insoluble glucans from the soluble chitosan.

The amount of chitosan was measured and the percent chitosan in the filtered solids was calculated to provide a % purity on a dry weight basis. The average molecular weight of the chitosan was measured by a size exclusion column (SEC) on chitosan that had been separated from the chitosan containing-material by acidifying with acetic acid and centrifugation.

First derivative ultraviolet spectrophotometry was used for measuring the degree of deacetylation of chitosan was first derivative ultraviolet spectrophotometry. This was described by Riccardo A. A. Muzzarelli and Roberto Rocchetti, Determination of the Degree of Acetylation of Chitosans by First Derivative Ultraviolet Spectrophotometry, Carbohydrate Polymers, 5:461-472, 1985.

The results of this example are in Table 1.

Example 2. Method of obtaining and purifying chitosan from fungal biomass using 12.8 % NaOH at greater than 0 PSIG.

40.8 kg of fungal biomass (*Aspergillus niger)* at 13.68 % dry solids, 11 liters of 50% NaOH, and 12 liters of water were added to a pressure reactor. This gave a final

ratio in the mixture of 8.0% dry biomass, 12.8 % NaOH, and 79.9% water. This alkali biomass solution was heated using a steam coil to approximately 130° C and held in the sealed reactor for 45 hours. Since this was above the boiling point of 12% caustic (104°C), 18-20 PSIG pressure was contained in the reactor, as well as the ammonia and other gases released in the associated reactions.

Samples were taken periodically. The samples were filtered and washed with water to remove the NaOH, salts and other soluble by-products. The filtered solids contained the chitosan-containing material, made up primarily of chitosan and glucans. The chitosan was then separated from the glucans by dissolving the filtered solids in acetic acid (pH 4.0), and centrifuging the insoluble glucans from the soluble chitosan.

Measurements were made in a similar manner to those described in Example 1. The results of this Example are in Table 2.

Example 3. Method of obtaining and purifying chitosan from fungal biomass using 30.1% NaOH at greater than 0 PSIG.

Chitosan was obtained and purified from *Aspergillus niger* using 30.1% NaOH. Other than a different caustic level, the conditions and processing steps are similar to those used in Example 2.

The results of this Example are shown in Table 3.

Example 4. Method of obtaining and purifying chitosan from fungal biomass using 24.9% NaOH at 0 PSIG.

208.6 kg *Aspergillus Niger* mycelium of which 18% was dry matter was mixed with 135 L of 50% NaOH to make a mixture that contained 24.9% NaOH and 8.9% solids. The mixture was heated to 110°C for the time periods indicated in Table 4, below. Analysis of products are reported in Table 4.

Example 5. Method of obtaining and purifying chitosan from fungal biomass using 30% NaOH at 0 PSIG.

254 kg *Aspergillus Niger* mycelium of which 18% was dry matter was mixed with 250 L of 50% NaOH to make a mixture that contained 30% and 7% solids. The mixture was heated to 118°C for the time periods indicated in Table 5, below. Analysis of products are reported in Table 5.

Table 1

20.1% NaOH						
Time (hr)	Temp (C)	Pressure (PSIG)	Average Molecular Weight of Chitosan	Average Molecular Number of Chitosan	%DA of Chitosan	Chitosan Purity in dry cake
4	130	14	169,104	48,350	79	43.0
12	132	14	232,964	45,637	82	82.8
16	130	16	201,681	41,401	85	91.4
20	132	14	178,736	37,254	86	92.1
24	129	14	142,814	33,144	88	97.1
28	130	14	122,975	28,540	89	98.4

PSIG* pounds per square inch gauge

Cake* refers to the dry solids remaining after the reaction

%DA* refers to percent deacetylation

Table 2

12.8% NaOH						
Time (hr)	Temp (C)	Pressure (PSIG)	Average Molecular Weight of Chitosan	Average Molecular Number of Chitosan	%DA of Chitosan	Chitosan Purity in dry cake
6	130	14	147,574	38,236	83	50.5
12	128	13	226,316	38,349	82	80.6
15	130	15	258,933	38,428	83	81.9
18	128	15	210,449	34,844	84	80.3
24	128	14	203,543	33,856	87	84.5
30	130	15	150,629	26,669	89	90.3
40	130	15	101,464	23,253	92	95.0
42	130	15	103,143	23,624	93	97.9
45	130	15	103,143	23,624	93	97.9

Table 3

30.1% NaOH						
Time (hr)	Temp (C)	Pressure (PSIG)	Average Molecular Weight of Chitosan	Average Molecular Number of Chitosan	%DA of Chitosan	Chitosan Purity in dry cake
2	131	14	206,647	61,876	89	53.0
4	135	11	176,844	50,253	90	76.9
6	133	10	152,997	42,720	91	80.2
8	133	10	134,026	38,885	92	87.8
10	132	13	115,210	34,949	93	85.5
12	132	11	107,080	32,099	94	89.7
14	132	10	100,386	29,954	93	94.6
16	132	10	89,452	29,416	94	96.9

Table 4
0 PSIG

24.9% NaOH

Time (hr)	Temp (C)	Average Molecular Weight of Chitosan	Average Molecular Number of Chitosan	%DA of Chitosan	Chitosan Purity in dry cake
3	109	106,615	44,690	73.8	15.3%
4	108	101,681	43,523	81.1	22.7%
6	108	99,004	42,855	82.4	27.1%
7	108	99,850	40,977	85.2	29.9%
8	112	91,112	39,815	84.9	35.9%
24	111	77,626	32,463	93.2	39.0%

Table 5
0 PSIG

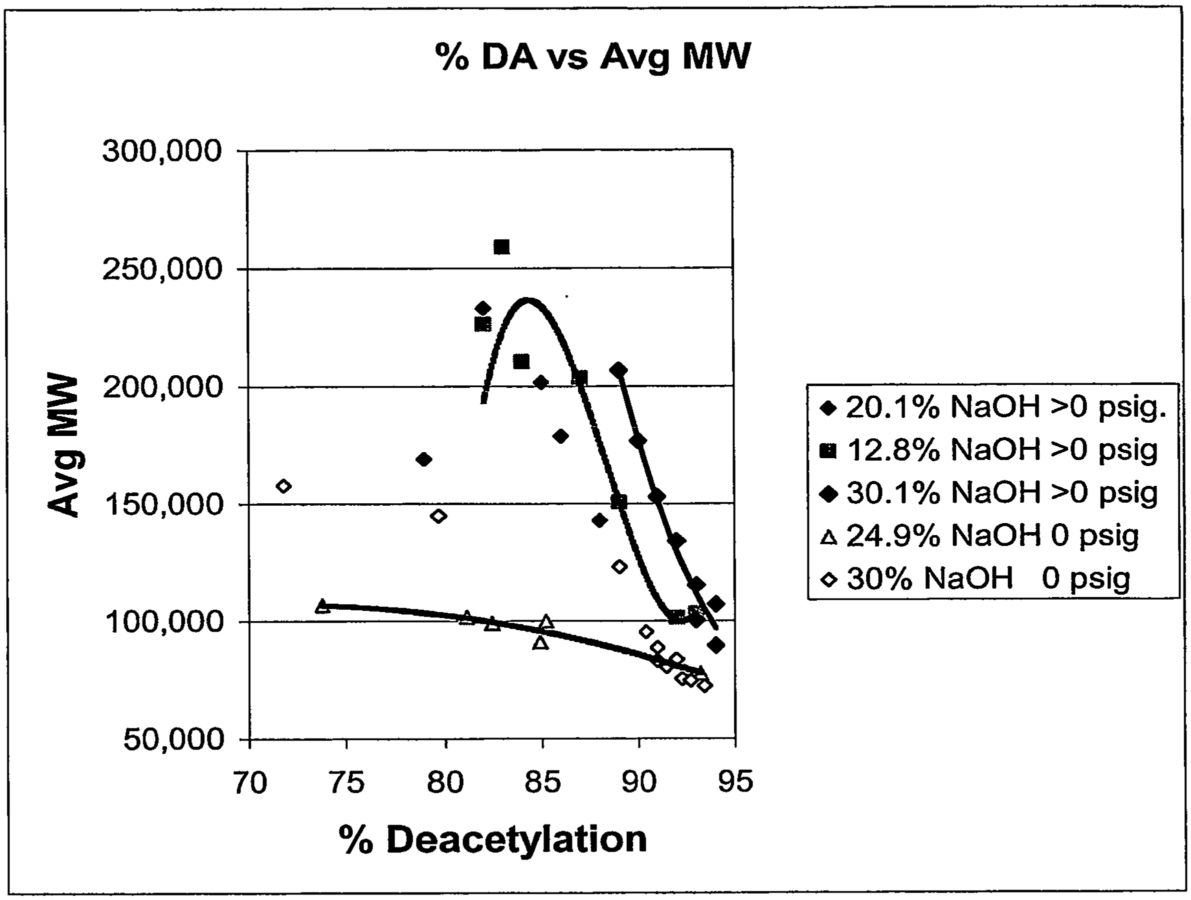
30% NaOH

Time (hr)	Temp (C)	Average Molecular Weight of Chitosan	Average Molecular Number of Chitosan	%DA of Chitosan	Chitosan Purity in dry cake
2	85	157770	81506	71.8	19.5%
4	105	144843	70204	79.7	25.8%
7	115	123054	58453	89.0	33.7%
12	119	95370	42371	90.4	57.9%
14	119	88609	39735	91.0	64.0%
16	117	83685	37011	92.0	65.6%
18	114	83104	35751	91.0	72.6%
20	115	80494	35611	91.5	74.2%
22	115	75438	32930	92.3	74.4%
24	117	74664	31919	92.7	75.4%
26	115	72383	31153	93.4	78.7%

The results provided above show that at pressures greater than 0 PSIG the molecular weight of chitosan is greater at a specific deacetylation level when compared with chitosan made at 0 PSIG at the same deacetylation level. Furthermore, it is expected that by maintaining constant pressure on the reaction, greater temperatures can be used while not depolymerizing the chitosan.

Graph 1 provided below presents a comparison of the average molecular weight to the percent deacetylation from Tables 1 through 5 above. The open symbols represent data collected at 0 PSIG, and the solid symbols represent data collected at pressures greater than 0 PSIG.

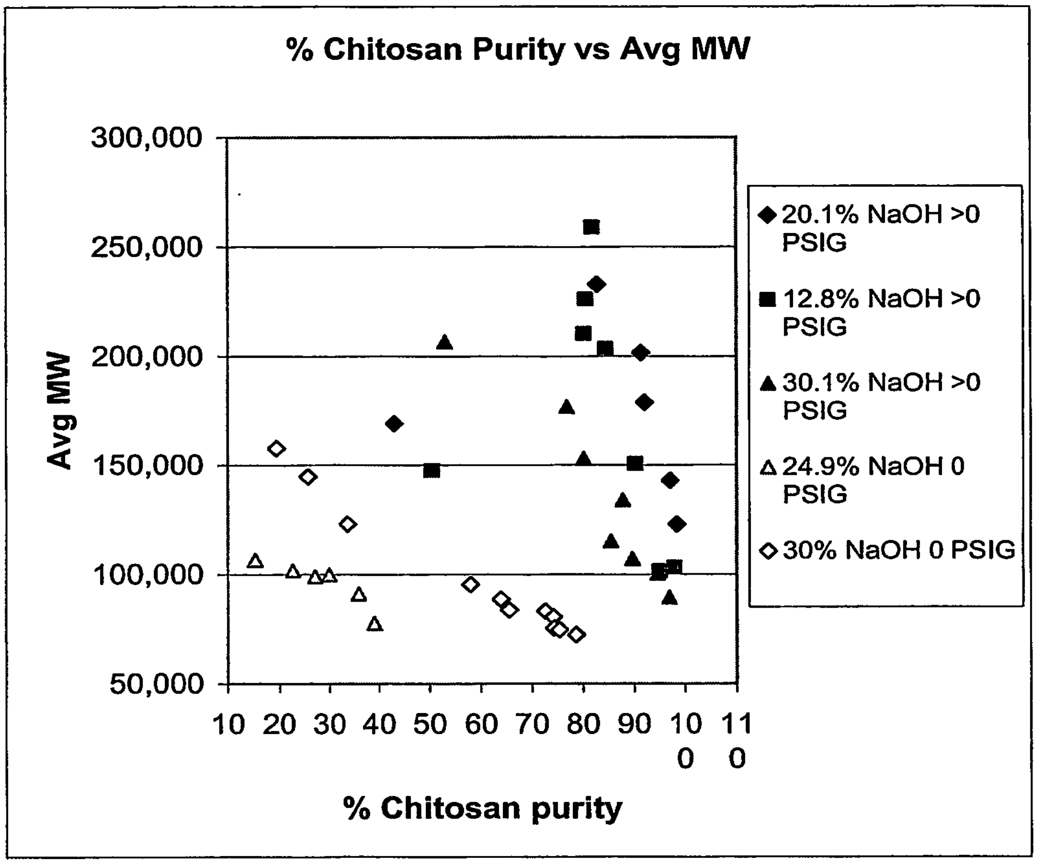
Graph 1



The results provided above also show that at pressures greater than 0 PSIG the molecular weight of chitosan is greater at a specific purity level when compared with chitosan made at 0 PSIG at the same purity level.

Graph 2 provided below presents a comparison of the average molecular weight to the percent purity of the chitosan from Tables 1 through 5 above. The open symbols represent data collected at 0 PSIG, and the solid symbols represent data collected a pressures greater than 0 PSIG.

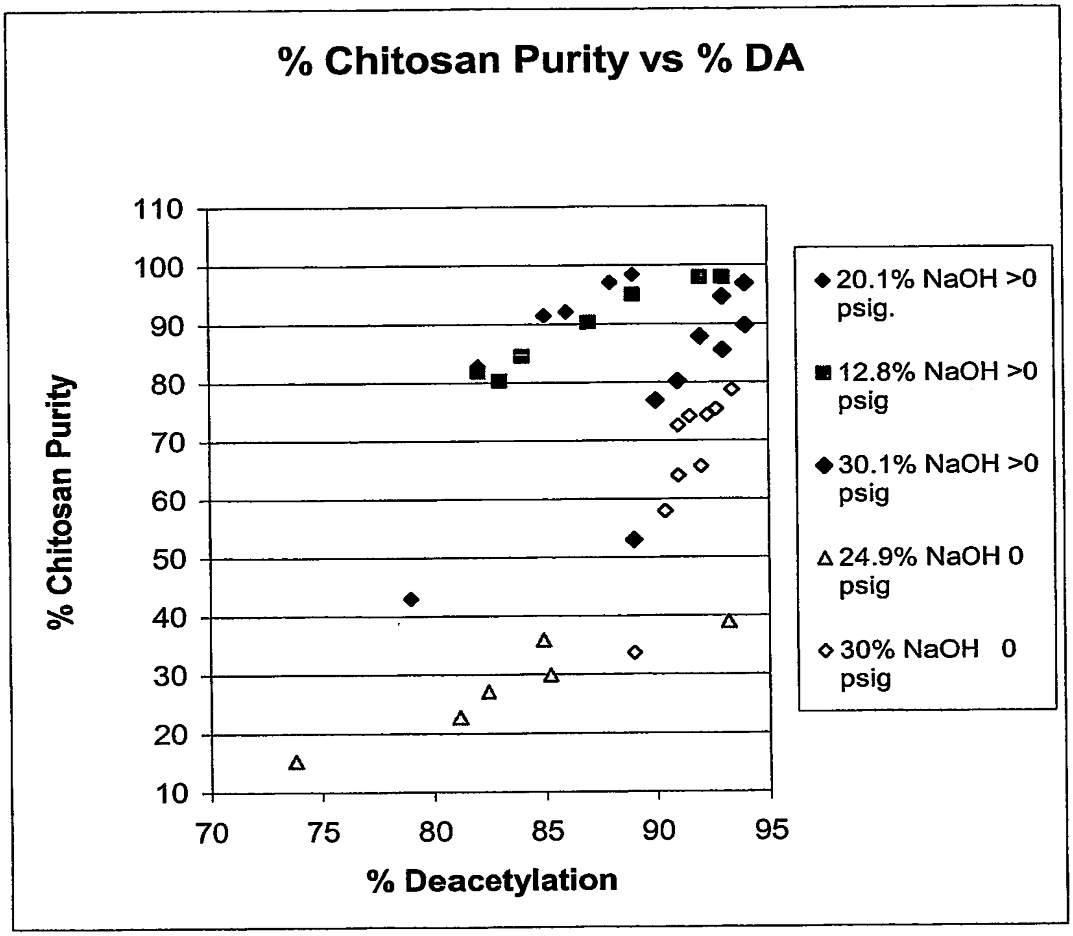
Graph 2



The results provided above also show that at pressures greater than 0 PSIG the average molecular weight is greater at higher percent purity of chitosan level when compared with chitosan made at 0 PSIG at the same purity level.

Graph 3 provided below presents a comparison of the percent purity of the chitosan to the percent deacetylation of the chitosan from Tables 1 through 5 above. The open symbols represent data collected at 0 PSIG, and the solid symbols represent data collected a pressures greater than 0 PSIG.

Graph 3



The results provided above also show that at pressures greater than 0 PSIG the percent purity of chitosan is greater at a specific percent deacetylation level when compared with chitosan made at 0 PSIG at the same purity level.

Having illustrated and described the principles of the invention in multiple embodiments and examples, it should be apparent to those skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications coming within the spirit and scope of the following claims.